CRISPR used to genetically edit coral, researchers report

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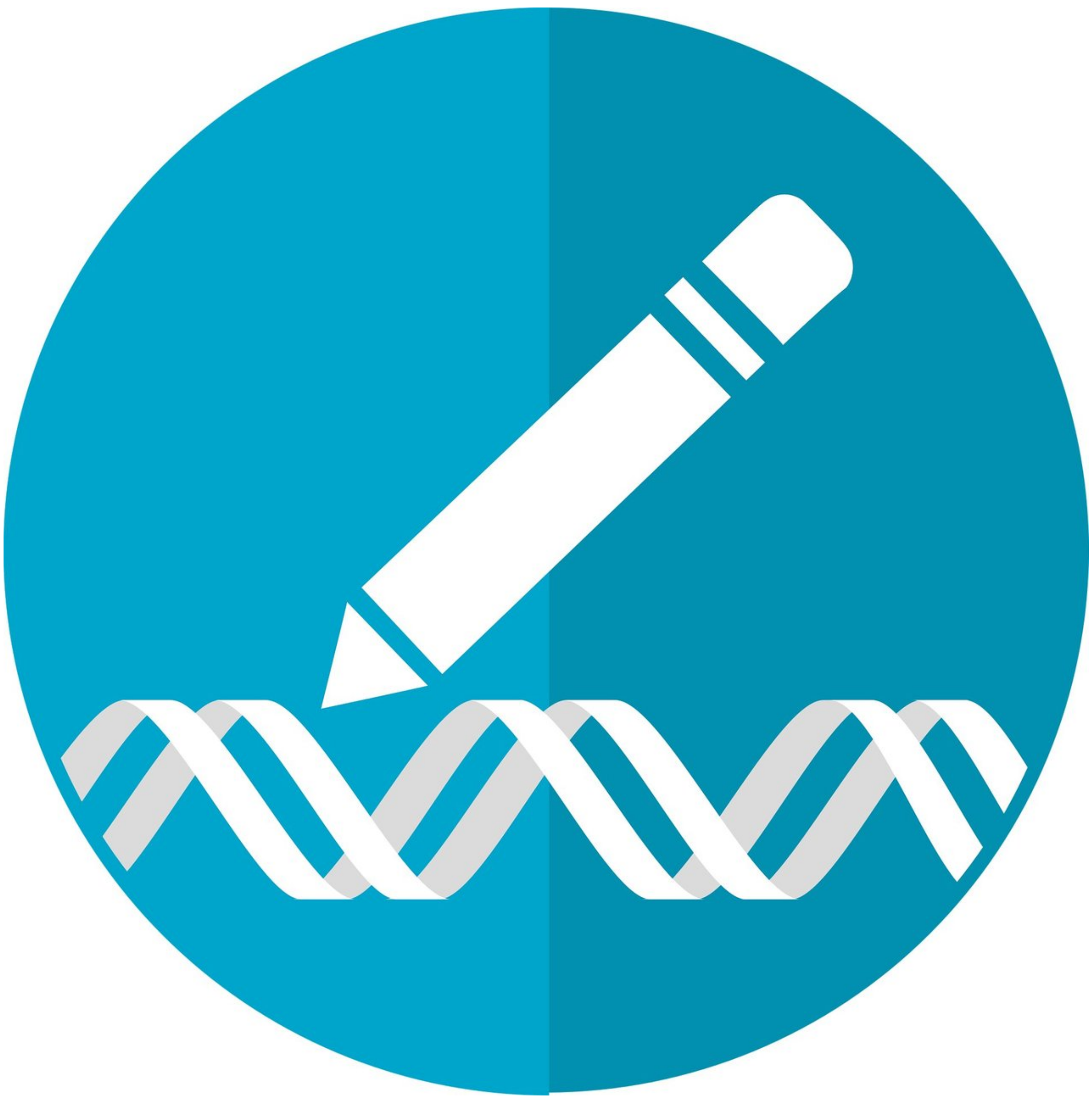

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Coral reefs on the precipice of collapse may get a conservation boost from the gene-editing tool known as CRISPR, according to researchers at the Stanford University School of Medicine and their collaborators.

The scientists found, for what appears to be the first time, definitive evidence that the CRISPR-Cas9 gene-editing tool could be a potent resource for coral biologists. Phillip Cleves, PhD, a postdoctoral scholar at Stanford, is a geneticist whose efforts to delineate gene function in animals resides squarely within the marine invertebrate realm—namely, corals.

"Up until now, there hasn't been a way to ask whether a gene whose expression correlates with coral survival actually plays a causative role," Cleves said. "There's been no method to modify genes in coral and then ask what the consequences are."

The study will be published online April 23 in the *Proceedings of the National Academy of Sciences*. Cleves is the lead author. John Pringle, professor of genetics at Stanford, and Mikhail Matz, PhD, associate professor of integrative biology at the University of Texas-Austin, share senior authorship.

The damage of coral bleaching

In the late 1990s, the ocean's coral reefs experienced the first big wave of something called coral bleaching, a bleak event in which ocean conditions—most prominently higher temperatures—kill off or "bleach" parts of the reef, turning once-vibrant colors bland and damaging the entire reef ecosystem.

Cleves' work, conducted in collaboration with researchers at UT-Austin and the Australian Institute of Marine Science, sprouted from a conversation at an international coral meeting that aimed to concretely understand the genes behind coral survival. Are there some genes that render corals more resilient to spikes in ocean temperatures? Or perhaps a gene that helps establish new coral colonies? Scientists had hypothesized answers to these questions, but to truly know, Cleves wanted to create a technique that could allow coral biologists to answer such questions more rigorously.

"We want to use CRISPR-Cas9 with the express interest to start understanding what genes are critical to coral biology," Cleves said.

CRISPR is a fast, effective tool that can be used to target and modify DNA sequences. "Breaking" genes to reveal the effects on the organism is a concept that's been the linchpin of decades of molecular biology. Now, CRISPR is helping speed up the process in many diverse animal models, but applying it to corals (don't be fooled—corals are animals, not plants) has proven tricky due in part to their infrequent reproduction. And until Cleves and his collaborators conducted this research, the use of the gene-editing tool had never been reported in corals.

"We hope that future experiments using CRISPR-Cas9 will help us develop a better understanding of basic coral biology that we then can apply to predict—and perhaps ameliorate—what's going to happen in the future due to a changing climate," Cleves said.

Spawning by moonlight

Corals pose a bit of a problem when it comes to CRISPR because of their spawning cycles. Most corals, including the Acropora millepora that was the focus of the study, breed only once or twice a year, during October and November in the Great Barrier Reef, cued by the rise of a full moon. During this fleeting window, corals release their sex cells into the ocean. When the eggs and sperm meet, they form zygotes, or fertilized single cells. During the narrow time window before these cells begin to divide, a researcher can introduce CRISPR by injecting a mixture of reagents into these zygotes to induce precise mutations in the coral DNA.

Retrieving the zygotes is quite a logistical challenge, Cleves acknowledged. Fortunately, his collaborators in Australia have the timing down pat; they can predict when the moon spawn will occur within a couple of days, allowing them to take coral samples from the reef to gather zygotes for experimentation.

Cleves traveled to Australia to begin experimenting with CRISPR, targeting three coral genes: red fluorescent protein, green fluorescent protein, and fibroblast growth factor 1a, a gene that is thought to help regulate new coral colonization.

Using CRISPR, the scientists made a type of genetic tweak that knocked out the genes, rendering them incapable of functioning. In the case of the red and green fluorescent proteins, determining if CRISPR worked would be easy—like seeing lights switch off. Or so they hoped. However, it turns out that there are multiple copies of red and green fluorescent-protein genes. So knocking out one copy didn't put a stop to the glow altogether.

"Although we are not sure we saw convincing loss of fluorescence, DNA sequencing showed us that we were able to molecularly target both the red and the green fluorescent protein genes," Cleves said. This showed the researchers that, in one go, CRISPR could successfully alter multiple genes if the two were similar enough—a boon to genetic manipulation, as genes are often duplicated during evolution.

As for the third gene, fibroblast growth factor 1a, which only has one gene copy, post-CRISPR sequencing showed success: in some embryos, the gene was largely mutated, suggesting that CRISPR will work well to modify single-copy coral genes.

Cleves said the ultimate goal is not to engineer a genetically resilient super-coral that could populate the ocean—such a feat is currently implausible and would raise significant ethical questions. "Right now, what we really want to do is figure out the basic mechanisms of how coral works and use that to inform conservation efforts in the future," he said. "Maybe there are natural gene variants in coral that bolster their ability to survive in warmer waters; we'd want to know that."

'An all-hands-on-deck moment'

Although the current work is a proof-of-principle study, now Cleves and others are beginning to tinker with genes that are more ecologically pertinent. And he hopes that others do the same.

"I want this paper to provide an early blueprint of the types of genetic manipulations that scientists can start doing with corals," Cleves said. In the next few years, he hopes to see other groups knocking out coral genes potentially involved in bleaching, skeletal growth, or the critical symbiosis with the algae that provide most of the corals' energy.

Today, as much as 27 percent of the global reef ecosystem has been lost to a combination of climate change and human activities—and Cleves is feeling the urgency.

"This is an all-hands-on-deck moment," he said. "If we can start classifying what genes are important, then we can get an idea of what we can do to help conservation, or even just to predict what going to happen in the future. And I think that makes this a really exciting time to be a basic biologist looking at the genetics of coral."

More information: Phillip A. Cleves el al., "CRISPR/Cas9-mediated genome editing in a reef-building coral," *PNAS* (2018). www.pnas.org/cgi/doi/10.1073/pnas.1722151115

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